Team iGEM UFSCar-Brasil

12% SDS PAGE

1. Set the casting frames (clamp two glass plates in the casting frames) on the casting stands.

2. Make the **separating gel** (2 gels)**:**

In a separate small beaker, pipette the following reagents: 3,1 mL of destilated water, 80µL SDS

10%, 3,2 mL Acrylamid 40%, 1,5 mL Tris-HCl 2M pH 8.8, adding 50 µL Ammonium Persulfate 10% and 10 µL TEMED last.

4. Swirl the solution gently but thoroughly.

5. Pipet appropriate amount of separating gel solution into the gap between the glass plates.

6. To make the top of the separating gel be horizontal, fill in isopropanol into the gap until a overflow.

7. Wait for 20-30min to let it gelate.

8. After polymerization, discard the isopropanol.

9. Make the **stacking gel**:

In another small beaker, mix the following reagents: 3,09 mL of destilated water, 40 µL SDS 10%, 600 µL Acrylamid 40%, 250 µL Tris-HCl 2M pH 6.8, 10 µL TEMED and 37,5 µL Ammonium Persulfate 10%.

10. Pipet in stacking gel untill a overflow.

11. Insert the well-forming comb without trapping air under the teeth. Wait for 20-30min to let it gelate.

12. Mix your samples with sample buffer (loading buffer).

13. Load prepared samples into wells and make sure not to overflow. Don't forget loading protein marker into the first lane.

14. Set an appropriate volt and run the electrophoresis when everything's done.